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Extraction of Fat Tissue from Meat Products with Supercritical Carbon Dioxide

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An alternative extraction method for the removal of fats from meat samples is described employing supercritical carbon dioxide as the extracting medium. Experiments conducted at 35–70 MPa and 80 °C have shown that dense CO_2 is an effective agent for selective removal of fat from a variety of meat matrices. Results obtained on meats varying in fat content from 2 to 35% by weight indicate that over 96% of the theoretical fat content can be removed. Rapid extraction fluxes are realized when comminution and dehydration are performed on the samples prior to extraction. The described technique generates minimal toxicological risk to laboratory personnel and eliminates disposal problems of residual solvents.

The extraction of lipophilic phases from natural products such as grains and tissue samples is a routine procedure in the assay for many specific analytes. An assortment of techniques have been developed to accomplish this task, including thermal rendering (FSIS, 1986), solvent extraction (Ellis, 1984; Nelson, 1975), and adsorption from a liquid phase (Mills, 1959; Holden and Marsden, 1969; Maxwell et al., 1980). The use of solvent extraction for the removal of fats is widely practiced in many analytical laboratories and can result in the accumulation of copious amounts of solvent in the workplace. In addition, many organic solvents are toxic, creating potential health hazards for associated laboratory personnel. Such problems are magnified in quality control and regulatory laboratories where many samples are regularly assayed by solvent extraction procedures.

Extraction of fats, i.e., triglyceride mixtures, can readily be accomplished with supercritical carbon dioxide (SC-CO_2), as demonstrated by a number of researchers (Peter and Brunner, 1978; Quirin, 1982; King et al., 1983; Fattori et al., 1987). Much of the reported research has been devoted to nonanalytical applications of the technology, such as the removal of oils from vegetable seeds (Friedrich et al., 1982; Stahl et al., 1980), spices (Taniguchi et al., 1987), and marine products (Yamaguchi et al., 1986). Delipidation of animal muscles has been cited in the literature (Hardartottir et al., 1987; Zosel, 1977), primarily as a processing aid in the food industry. In this study, we have utilized the solvent properties of dense CO_2 to effect the removal of fats from meat matrices.

The use of supercritical extraction (SCFE) in tandem with supercritical fluid chromatography (SCFC) and other instrumental techniques has been reported recently by a number of investigators (Stahl, 1977; Unger and Roumeliotis, 1983; Sugiyama et al., 1985; Hawthorne and Miller, 1987; Gmur et al., 1986, 1987). The described methodology has been largely accomplished on a microscale, using small extraction tubes or cartridges (1–10 mL) to facilitate transfer into chromatographic instrumentation. Whereas this approach has been qualitatively applied to characterize extracts from a variety of samples (Engelhardt and Gross, 1988; Wright et al., 1987; Hawthorne et al., 1988), there have been no reported studies on the removal of lipids from biological samples, such as meats. The described research represents our initial attempts to develop a technique for extracting relatively large quantities of fat from meat products required in established regulatory protocols for pesticide residue analysis (FSIS, 1986).

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EXPERIMENTAL SECTION

Apparatus. The extractions were performed with the apparatus shown in Figure 1. Carbon dioxide from a cylinder (A) was fed to a compressor (C), (Model AGT-62/152-C; Haskel Engineering Corp., Burbank, CA), through a check valve (CV) and 5-μm particulate filter (F). Extraction pressures were set to the desired value by adjusting the air intake valve to the compressor in tandem with the setting on the downstream relief valve (RV). This arrangement permitted the extractor pressure to be regulated to ±1.4 MPa. The gas is admitted into the extraction tube by a series of valves (SV-1, SV-2, SV-3, SV-4), which permits gas flow from either end of the extraction vessel. The extraction tube is mounted vertically in a Hewlett-Packard 7610 gas chromatograph oven. The extraction gas is equilibrated to the oven temperature by passing it through a 3-m coil (either HC-1 or HC-2). Extractor tubes consist of 316 SS tubing (Part no. 15-099; Autoclave Engineers, Erie, PA) pressure-rated to 76 MPa at room temperature, with dimensions of 1.75 cm (i.d.) × 30.5–56 cm. Interconnecting lines between the major components of the extractor consisted of 316 SS, 0.32-cm-o.d., 0.159-cm-i.d. tubing rated to 80 MPa at 93 °C. Pressure gauges (PG-1, PG-2) were used to monitor the extraction pressure and the pressure drop across the extraction tube. The oven temperature was assessed by thermocouple (TC-1) while the extractor tube temperature was monitored by TC-2.

The solute-laden fluid was next passed through a micrometering valve (MV) to a receiver vessel. The receiver vessel, a modified 300-mL Magnedash autoclave (Part No. 70-1395; Autoclave Engineers), was held slightly above atmospheric pressure and could be heated if desired. The stirring assembly of the autoclave was removed, and the portals were fitted with a thermocouple, a gas delivery tube to the bottom of the autoclave, and a dip tube for sampling. Plugging of the micrometering valve, due to precipitation of the fat upon decompression of the supercritical CO_2, was eliminated by heating the valve. Flow rates were monitored downstream of the MV by a Fischer-Porter Model 10A 3355 flow meter (FM), calibrated for standard liters per minute of carbon dioxide. Total volume of CO_2 passed as a function of time was determined on a Singer Model DTM-200 gas totalizer (GT).

Sample Preparation. The meat samples utilized in this study were obtained from a local abattoir or grocery store. Initial extraction studies were performed on porcine lard and link sausage. Canned meat products, such as a homogenized ham-based luncheon meat, a smoked ham, and a low-fat import ham, were also extracted with SC-CO_2. Comminution of the meat samples was done with a Varco electric grinder. The moisture content of the meat was reduced by overnight drying in a Petri dish at room temperature. Moisture levels of 2.45%, 2.26%, and 1.80% by weight were achieved for the smoked ham, import hams, and luncheon meat, respectively, by this drying procedure. Crude fat and moisture were determined on the original, tempered, and extracted meat samples by standard AACC methods (AACC, 1983). Lard samples (14–20 g) for extraction were melted and placed on a glass wool support encased in a Teflon sleeve, before

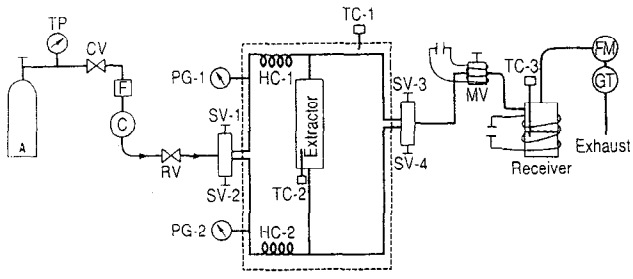

Figure 1. Experimental apparatus for supercritical fluid extraction of meat samples.

insertion into the extractor tube. Similarly, meat samples were placed on the Teflon sleeve or into the extraction tube directly.

Extraction Procedure. The extraction tube containing the meat sample was inserted into the extraction module and gradually brought to the appropriate temperature and pressure before commencement of gas flow. All extractions were performed at 80 °C to increase the solubility of the triglycerides in SC-CO_2 (Friedrich et al., 1982). Compression levels of 34.5 and 69 MPa were used to remove the fat from the meat matrix. The receiver vessel was held at atmospheric pressure and a temperature 10–20 °C below the extractor temperature to enhance the precipitation of the oil and to permit gas flow through the collection vessel. Flow rates of SC-CO_2 varied, depending on the meat matrix being extracted, but were typically between 10–20 standard L/min at ambient conditions.

Initially, experimental data were collected every 2–3 min, due to high extraction fluxes of fat from the meat matrix. In the case of longer extraction experiments, these intervals were extended, due to the reduction of solute flux into the supercritical fluid gas stream. Data collection consisted of recording the extraction pressure and temperature, receiver temperature, gas flow, and totalizer values at specific times. In addition, the oil captured in the receiver vessel was removed via a sampling tube, by opening a valve at the top of the receiver vessel. The fat was collected in a bottle and weighed at these same sampling times.

The time required to complete a SC-CO_2 extraction of a meat product was found to depend on the mass of the sample, sample fat and moisture contents, and the experimental conditions. Most experimental runs were terminated when over 95% of the available fat had been extracted or sample flux rate approached zero. Precision levels for the extraction of bulk lard samples were found to be 1.85% (relative standard deviation). The extraction flux was assessed at each sampling interval by converting the accumulated gas volume to grams of CO_2 and computing the weight fraction of solute contained in the total mass of solute and extraction gas. Fat and moisture analyses were performed on all samples before and after extraction.

RESULTS AND DISCUSSION

Initial experiments were conducted with a lard sample to test the effect of gas pressure on the rate of extraction. As shown in Figure 2, extraction at 69 MPa (10 000 psig) permitted 84.5% of the total lard sample to be extracted in 49 min. A reduction in the extraction pressure to 34.5 MPa (5000 psig) resulted in a much lower rate of fat removal as indicated by the slopes of the percent fat extracted versus extraction time in Figure 2. The time required to extract the lard sample at the lower pressure was almost tripled for a marginal improvement in total fat yield (91.4%).

Similar tests were performed on a link sausage matrix at both 34.5 and 69 MPa. For two of the extraction runs, the sausage was ground to assure the fat was uniformly distributed. The extraction results are shown in Figure 3, where the fat extracted (percent based on the total sample weight) is plotted versus the total weight of CO_2 passed through the extractor vessel. For the ground sausage samples, extraction at 69 MPa removes the fat from the protein matrix much more rapidly then at a 34.5-MPa compression level. This marked difference in solubilization

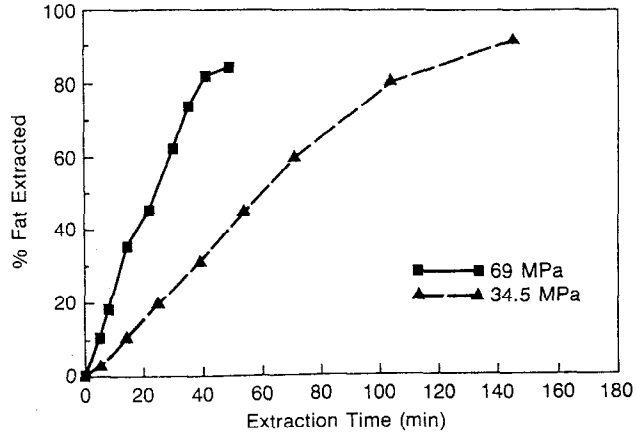

Figure 2. Supercritical carbon dioxide extraction of porcine fat.

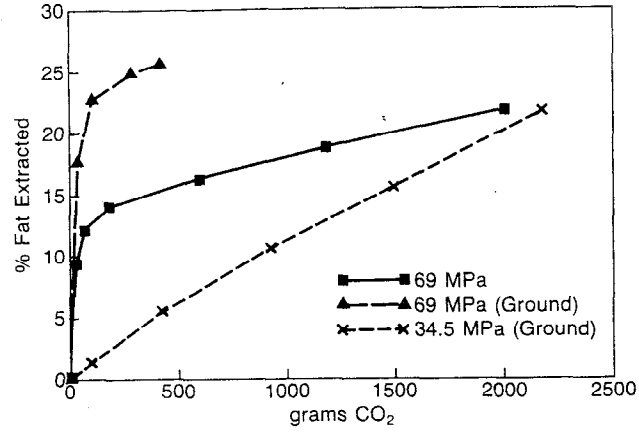

Figure 3. Fat removal from link sausage by SC-CO_2 at various pressures.

can be attributed to the enhanced solubility of the component triglycerides in CO_2 at higher gas pressures (Friedrich et al., 1982; Stahl et al., 1980). It is also apparent from Figure 3 that homogenization of the meat sample permitted more rapid extraction of the fat phase as evidenced by the slow rate of extraction of the unground sample at 69 MPa after passage of 2000 g of SC-CO_2.

The fat extracted for the experimental runs given in Figure 3 is below the determined amount of fat contained in the sausage sample, 38.3% by weight. To further confirm the importance of sample comminution on the extraction yield, the unground sample extracted previously at 69 MPa was ground in the Varco grinder and reextracted with CO_2 at the same pressure. This action resulted in a fat yield equivalent to 36.5% of the sample weight, an amount close to the previously quoted theoretical yield. As a result of this finding, all meat samples were subjected to extensive grinding to expose lipid-containing regions before the commencement of SC-CO_2 extraction.

Initial attempts to remove the fat phase from meat products having a low fat content and high moisture level (50–75% by weight) were thwarted despite rigorous grinding of the sample matrix. Fat yields for the ham-based luncheon meat and low-fat hams extracted at 69 MPa were poor, and the rate of extraction was slow despite the use of the higher extraction pressure. A considerable improvement in fat yield was realized by dehydrating the samples before extraction. For example, a smoked ham containing 2.80% fat, which had its moisture content reduced from 73.4 to 33.71 wt %, was found to contain only 1.06% of fat in the dehydrated product after extraction. Extended dehydration of a similar sample initially con-

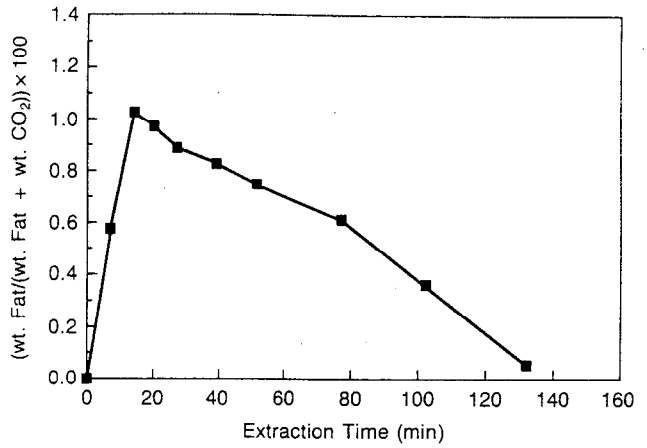

Figure 4. Weight fraction of fat from dehydrated luncheon meat in the supercritical fluid phase as a function of extraction time.

taining 3.31% fat resulted in a 0.72% level of residual lipids after extraction, when the moisture level had been being reduced from an initial value of 73.6 wt % to 1.52% by weight in the final extracted product. It would appear that the high water content of those meats inhibits SC-CO_2 contact with the lipid phase in the meat sample.

The rate at which the fat phase is removed from the meat sample by SC-CO_2 can be determined by computing the weight fraction of the extracted fat contained in the compressed gas phase at discrete time intervals. An example of this concept is shown in Figure 4 on a dehydrated ham-based luncheon meat sample, which was extracted with CO_2 at 34.5 MPa and an average flow rate of 16.8 L/min (measured at ambient conditions). As shown in Figure 4, there is a large increase in the fat content of the supercritical fluid phase during the initial 20 min of the extraction. The fat content in the supercritical fluid phase begins to gradually decrease after this initial extraction period and approaches zero afer 2 h of processing time. Similar trends for fat removal as a function of time have been recorded for the other meat samples extracted in this study.

The results reported in Figure 4 indicate that rapid extractions of the fat phase contained in meat samples can be effected with SC-CO_2. The time required to perform these extractions will depend not only on the pressure or temperature at which the extraction is conducted but on the quantity of lipid matter in the sample, the weight of sample taken for extraction, and the flow rate of the extraction gas. As indicated in Figure 4, fat solubilities at 34.5 MPa and 80 °C range between 0 and 1.0%, in agreement with equilibrium data for mixtures of triglycerides in SC-CO_2 under the same extraction conditions (Friedrich et al., 1982). Hence, it is important to provide a high flow of the extraction gas to ensure rapid depletion of the fat phase from the protein matrix.

An example of optimizing the extraction conditions for a specific meat product is given in Figure 5. Here an imported ham containing initially 1.86% fat and 73.4% water by weight was treated with SC-CO_2 at 34.5 MPa with an average flow rate of 17.3 L/min. The sample was ground and dehydrated prior to extraction with CO_2. The objective of performing this extraction was to obtain a multigram extract for subsequent sample workup prior to electron capture gas chromatographic analysis for pesticide residues. A large sample of ham (241 g) was taken for the extraction to obtain the desired quantity of fat, as well as to assure a representative sample of the meat product. The results presented in Figure 5 show that almost all of the fat contained in the sample was removed in 40 min,

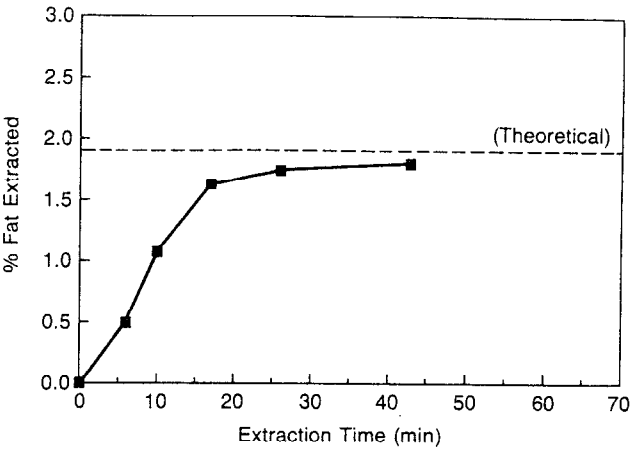

Figure 5. Rapid extraction of imported ham sample with SC-CO_2 at 34.5 MPa and 80 °C.

Table I. Fat Extraction Results on Various Meat Products

sample type	pressure, MPa	sample wt, g	fat yield, %	% fat extr
link sausage	69.0	52.87	20.25	99.6
luncheon meat	34.5	109.13	21.47	98.9
smoked ham	34.5	169.47	7.35	96.6
imported ham	34.5	241.19	4.37	97.3

yielding 4.37 g of fat for analysis.

Typical extraction results obtained on the meat products utilized in this study are presented in Table I. Most of the listed samples were extracted at 34.5 MPa, despite the reported higher extraction fluxes that can be obtained at 69 MPa. The lower extraction pressures were used primarily to test the feasibility of performing the extractions with lower pressure-rated equipment, thereby decreasing the cost of the experimental apparatus. Table I shows that almost a 5-fold range in sample size can be processed by this extraction method. The corresponding fat yields for the quoted sample weights in Table I are more than adequate for subsequent analysis of trace analytes contained in these fat matrices. The degree of fat removal is impressive, as judged by the percentage of available fat extracted from the samples in Table I.

In summary, the described technique is capable of removing fat from a variety of meat products under mild thermal conditions. Full fat extractions can be performed at a 34.5 MPa-gas compression level with high efficiency on low-fat-containing samples within 1 h. The supercritical fluid-based process achieves maximum results on finely comminuted and dehydrated meat samples, yielding over 96% of the theoretical fat content of the extracted samples. These encouraging results suggest that extraction with SC-CO_2 is a viable technique for replacing conventional solvent extraction procedures, thereby eliminating the generation of large quantities of residual solvents and their attendant disposal problem.

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